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Meyers et al.

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[54] **WEIGHT CONTROL METHOD**

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[52] **U.S. Cl.** **514/176**; 514/278; 514/282

[58] **Field of Search** 514/169, 176, 278, 282; 260/397

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,309,362	3/1967	Meyer et al.	260/397
3,340,248	9/1967	Meyer et al.	260/397

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Attorney, Agent, or Firm—Senniger, Powers, Leavitt and Roedel

[57] **ABSTRACT**

A method of controlling weight in a mammal comprising administering to the mammal a weight control agent for inhibiting weight gain. The weight control agent may comprise certain azine, thiosemicarbazone, or N,N'-disubstituted thiourea derivatives of nonpeptide opioid antagonists.

6 Claims, 4 Drawing Figures

FIG. 1

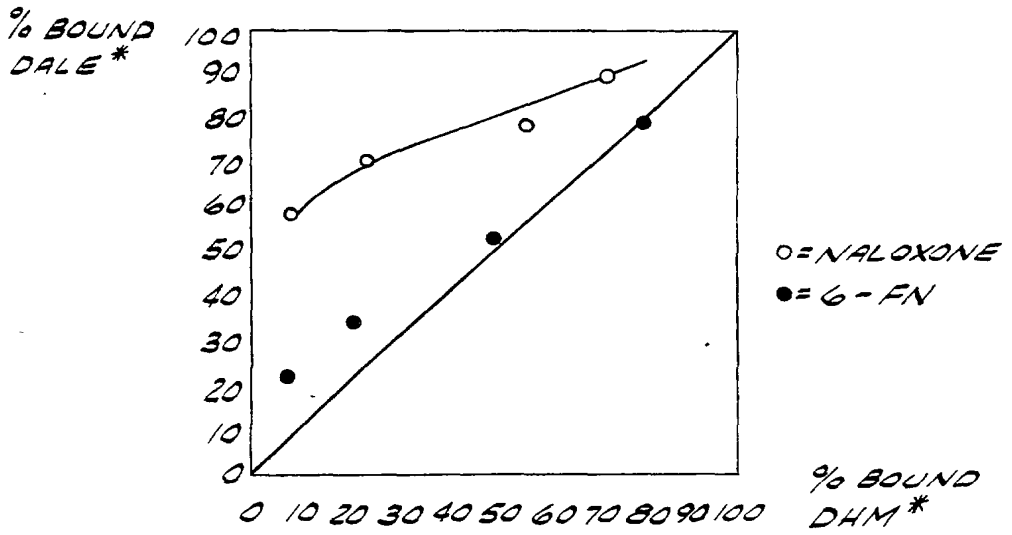


FIG. 2

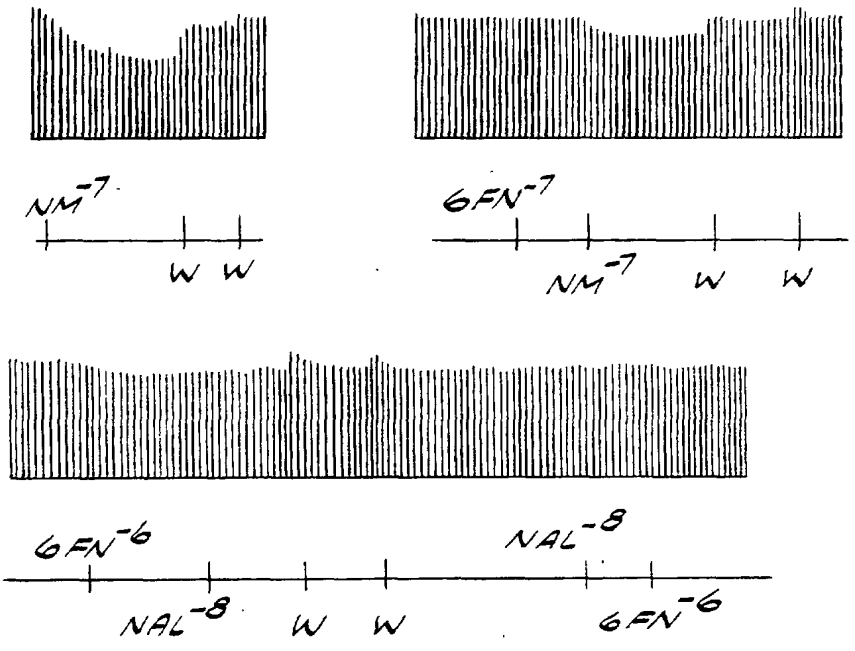


FIG. 3

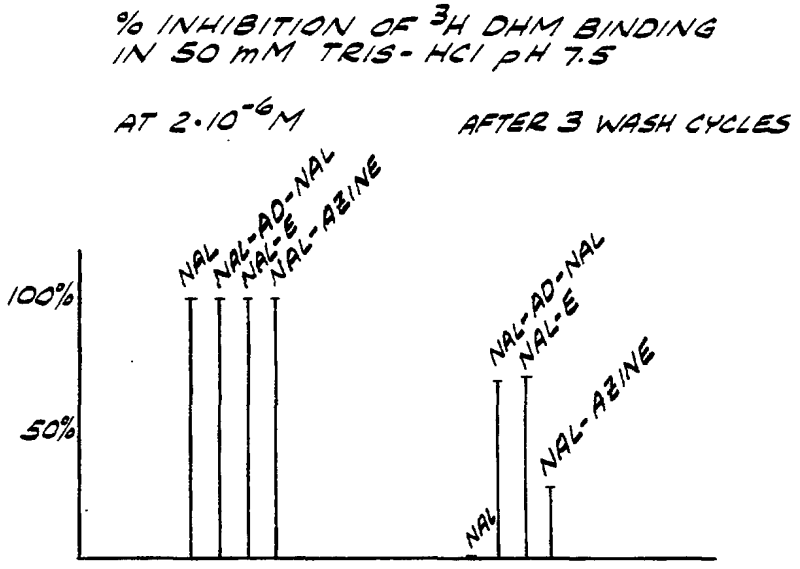
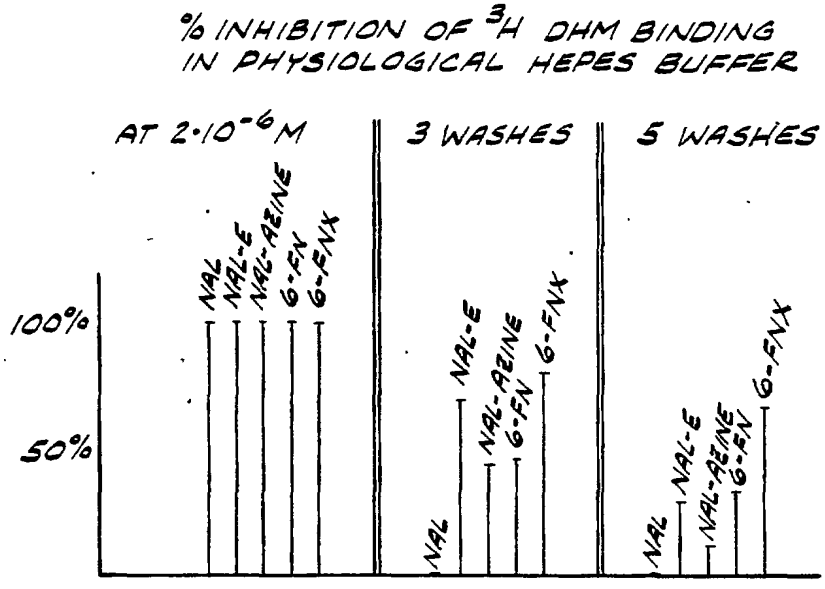


FIG. 4



WEIGHT CONTROL METHOD

BACKGROUND OF THE INVENTION

This invention relates to the field of weight control, and more particularly to a novel method for inhibiting weight gain in a mammal by administration to the mammal of a long acting weight control agent comprising a nonpeptide opioid antagonist and another moiety.

Previous studies have suggested that endogenous opioids may be involved in the control of feeding and appetite. See, for example, H. N. Bhargava, "Opiate Agonists and Antagonists: Pharmacological, Behavioral, and Neurochemical Effects of Stereoisomers," in "CRC Handbook of Stereoisomers: Drugs in Psychopharmacology", D. F. Smith, Ed., CRC Press, Boca Raton, Florida, 1984, pp. 401–439, and the references cited therein. In particular, it has been demonstrated that the administration of naloxone reduces food intake in animals (for example, mice, rats, guinea pigs, squirrel monkeys) and humans by blockage of opiate receptors, Bhargava, supra; Herman and Holtzman, *Life Sci.*, 34, 1–12 (1984). Yim and Lowy, "Opioids, Feeding, and Anorexias", *Federation Proceedings* Vol. 43, 14, pp. 2893-2897 (November 1984), reported that administration of the opioid antagonists naloxone and naltrexone were effective in suppressing feeding that had been induced by administration of 2-deoxy-D-glucose. The latter paper further reported that naloxone was effective in reversing anorexia induced by opioid agonists such as morphine.

In the development of compositions and methods for weight control, there is a need for anorexic or other weight control agents which are long lasting, generally non-toxic, and substantially free of any other adverse side effects. There is a particular need for compositions which may be administered orally to produce persistent control of weight without toxic effects on the recipient.

Hahn, Pasternak et al, *J. Neuroscience*, 2, 572-576 (1982), discovered that the opiate antagonist naloxone azine has a long lasting effect, i.e, that it is removed only very slowly after having been bound to the sites of opioid receptors. However, Hahn et al did not disclose any possible use of naloxone azine as a weight control agent.

SUMMARY OF THE INVENTION

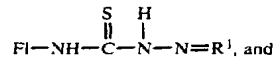
Among the several objects of the present invention, therefore, may be noted the provision of an effective method for inhibition of weight gain in mammals; the provision of such a method which provides a persistent and even effect on food intake and utilization; the provision of such a method which produces no significant toxic or other unfavorable side effects on the recipient; the provision of such a method in which a weight control agent is administered orally to the recipient; and the provision of novel compositions useful in such method of inhibition of weight gain.

Briefly, therefore, the present invention is directed to a novel method for controlling weight in a mammal. In this method, a weight control agent for inhibiting weight gain is administered to the mammal, the agent being selected from among:

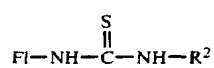
$$A{=}N{-}N{-}R^1 \qquad \text{(Formula I)}$$

$$X{=}N{-}N{=}Y{=}N{-}N{=}R^1 \qquad \text{(Formula II)}$$

-continued



(Formula III)



(Formula IV)

where R^1 comprises a divalent nonpeptide opioid antagonist, R^2 comprises a monovalent nonpeptide opioid antagonist, A is selected from among steroid moieties and fluorescent moieties, X is selected from among nonpeptide opioid antagonists and fluorescent moieties, Y comprises a steriod moiety, and Fl comprises a fluorescent moiety.

The invention is further directed to a weight control preparation adapted for administration to a mammal for inhibition of weight gain. The preparation comprises a weight control agent selected from among Formulae I to IV, above, and a pharmaceutically acceptable carrier, excipient or adjuvant.

The invention is also directed to compounds corresponding to Formulae II and IV; and further to compounds corresponding to the formula

$$A^1{=}N{-}N{=}R^1 \qquad \text{(Formula VII)}$$

where A^1 comprises a steroid moiety.

Other objects and features will be in part apparent and in part pointed out hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a plot comparing the extent of displacement of two different radiolabeled indicator ligands in opioid receptor site selectivity tests conducted on 1-(N)-fluoresceinyl naloxone thiosemicarbazone ("6-FN").

FIG. **2** illustrates the agonist effect of normorphine in inhibiting the contraction of guinea pig ileum; the antagonist effect of 6-FN with respect to normorphine; the slight agonist effect of 6-FN; and the antagonist effect of naloxone with respect to 6-FN.

FIG. **3** is a bar graph illustrating the relative persistence of binding of naloxone and certain of the weight control agents of the invention to the opiate receptors in rat brain cells in 50 mM Tris-HCl pH 7.5 buffer, as determined by the effect on inhibition of ^{3}H-dihydromorphine (^{3}H-DHM) binding which results from repetitive washing of a rat brain preparation to which the opioid had been attached.

FIG. **4** is a bar graph similar to FIG. **3** for ^{3}H-DHM displacement tests carried out in HEPES buffer.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

In accordance with present invention it has been discovered that long lasting suppression of weight gain can be achieved by administration to a mammal of a weight control agent comprising certain azine, thiosemicarbazone, or N,N'-disubstituted thiourea derivatives of nonpeptide opioid antagonists. Particular compounds which are effective for this purpose include hybrid (mixed) azines of an opioid antagonist and a steroid:

$$A^1{=}N{-}N{=}R^1 \qquad \text{(Formula VII)}$$

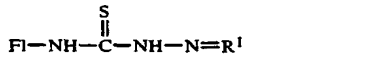
where A^1 comprises a steriod moiety and R^1 is a divalent nonpeptide opioid moiety; mixed azines of an opioid antagonist and flouorescent moiety:

$$Fl=N-N=R^1 \quad \text{(Formula VIII)}$$

where Fl comprises a fluorescent moiety; sterioid bis-(opioid azine)s and other mixed diazines:

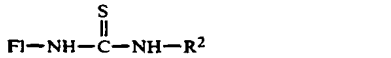
$$X=N-N=Y=N-N=R^1 \quad \text{(Formula II)}$$

where Y comprises a steroid moiety and X is either an opioid or fluorescent moiety; thiosemicarbazones of an opioid antagonist and a fluorescent moiety:



(Formula III)

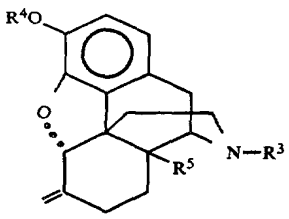
where Fl comprises a fluorescent moiety, and thioureas that are N,N'-substituted with a monovalent nonpeptide opioid antagonist and a fluorescent moiety:



(Formula IV)

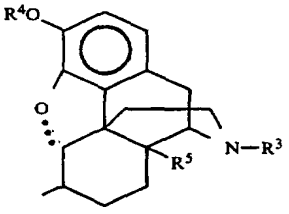
where R^2 is a monovalent nonpeptide opioid moiety.

It is preferred that the opioid moieties R^1 and R^2 comprise a normorphinan, normorphine (4,5-epoxymorphinan), or benzonormorphinan structure, most preferably a morphine structure corresponding to the formula



(Formula V)

or



(Formula VI)

where R^3 comprises hydrogen, alkyl, alkenyl or an alicyclic substituent, R^4 comprises hydrogen, alkyl or acyl, and R^5 comprises hydrogen, hydroxyl or acyloxy. Most preferably, R^4 comprises either hydrogen or methyl, R^5 hydroxyl, and R^3 either allyl or cyclopropylmethyl.

In accepted in vitro tests for determining binding characteristics, bis(opioid) azines corresponding to the formula

$$R^1=N-N=R^1 \quad \text{(Formula XVI)}$$

have been demonstrated to be significantly longer acting than the corresponding hydrazones having the formula

$$R^1=N-NH_2 \quad \text{(Formula XVII)}$$

i.e., the bis(opioid) azines are more firmly attached to opioid receptor sites and thus more difficult to remove from the sites by leaching or washing. In part this effect may be attributable to the presence of two opioid moieties in the bis(opoid) molecule, as a result of which this molecule may be able to bind to more than one receptor sites at once, thereby greatly enhancing the affinity and decreasing the rate of dissociation.

Surprisingly, however, it has been discovered that an affinity for the receptors sites even greater than that of the bis(opiod) azines is exhibited by the various hybrid compounds utilized in the weight gain control method of the invention. Based on in vitro tests, all of the weight control agents used in the novel method show advantageously long acting binding characteristics in conventional rat brain preparations. Especially strong binding affinity is exhibited by the thiosemicarbazones of opioid antagonists and fluorescent moieties of Formula III. These are not only very long acting with respect to the receptor sites of rat brain synaptosomal preparation but they are also long acting when bound to guinea pig ileum. Accordingly, the method of the invention is believed to provide the basis for a highly effective and superior method for the control of weight in mammals.

The mechanism by which opioid antagonist operate to control weight gain is not fully understood in the art. To some extent, the antagonist may act as an anorexic agent, i.e. an agent which suppresses appetite. Alternatively, the agent may act in some way to reduce the volume of food intake without any subjectively experienced effect on the appetite. It is also believed that opioid antagonists may affect either the metabolism of the recipient or its selection of food in a way which reduces or inhibits the gain of weight.

Regardless of the exact biological mechanism by which weight is controlled, it is believed that the biological activity of the antagonist, and thus the weight control effect achieved, depends on binding of the agent to opioid receptor sites in the mammal's system. Thus, long acting binding characteristics are desirable because they prolong the effect achieved with a given dosage, tend to reduce the total intake of opiate required for weight control, and further tend to even out the biological impact of the administration of the weight control agent.

No definitive data has been developed to explain why the compounds comprising the weight control agents of the invention exhibit such persistent binding to opiate receptor sites. It is believed that upon binding, the weight control agent molecule undergoes some change in conformation, and that this may result in "locking" of the molecule to the site because of steric factors relating to the substantial size of the other moiety or moieties in the molecule. In this regard, it is preferred that the fluorescent or steroid moiety present in the molecule have a linear dimension of at least 15 to 16 angstroms in at least one direction. Although this criteria provides a basis for selection of weight control agents other than those specifically disclosed herein, it does not fully explain all of the advantageous properties of the weight control agents of the invention, particularly the fluores-

cent/ opioid thiosemicarbazones which exhibit superior binding to both rat brain preparation and guinea pig ileum.

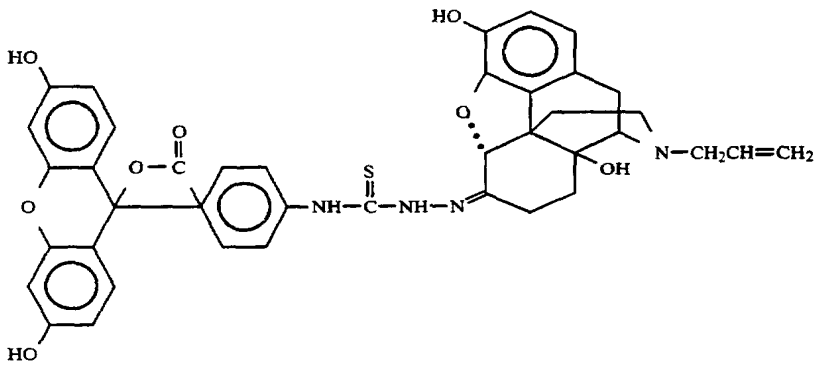
Where the weight control agent comprises a fluorescent moiety, it may further be used in receptor site studies as described in the copending and coassigned application of Vera Kolb Meyers, "Fluorescent Opioid Reagents and Methods of Use Thereof in the Detection of Opioid Receptors", Serial No. 621,384, filed June 18, 1984. Thus, in this instance the weight control agent may be used not only therapeutically for the suppression of appetite, but also in the analysis of a patient's apparent need for weight control therapy as potentially affected by the distribution of receptor sites in his system. Moreover, the fluorescent labeled moiety may be used in the evaluation of its own effectiveness in long lasting blocking of opioid receptor sites for purposes of weight control. Where a hybrid fluorescent/opioid azine, disubstituted thiourea, or thiosemicarbazone is used in vivo for diagnostic purposes or for research, it may be desirable to label it with a radioisotope as described in the aforesaid application of Vera Kolb Meyers, Serial No. 621,384. In such application, it is particularly preferred that a fused ring fluorescent moiety be used and that it be labeled either with carbon-14, nitrogen-15 or iodine-131.

Within the above described parameters, a variety of fluorescers can constitute the fluorescent moiety. Effective long acting binding has been demonstrated with fluorescent moieties which are highly water soluble (such as for example G-orange), of modest water solubility (rhodamine), and of very low water solubility (fluorescein). Weight control agents containing any of these fluorescers are effective in accordance with the method of the invention. It may be possible that the pharmacodynamic mechanism of the weight control agent varies with water solubility. Since there are opioid receptor sites in both the brain and the gut, it may be that relatively water soluble agent, such as those containing G-orange, may work primarily by attachment to the sites in the gut, while relatively insoluble agents may more readily pass the blood/brain barrier and attach to the sites in the brain. In the former case the effect on food intake and utilization may be relatively direct, while in the latter the effect may be manifested through the central nervous system.

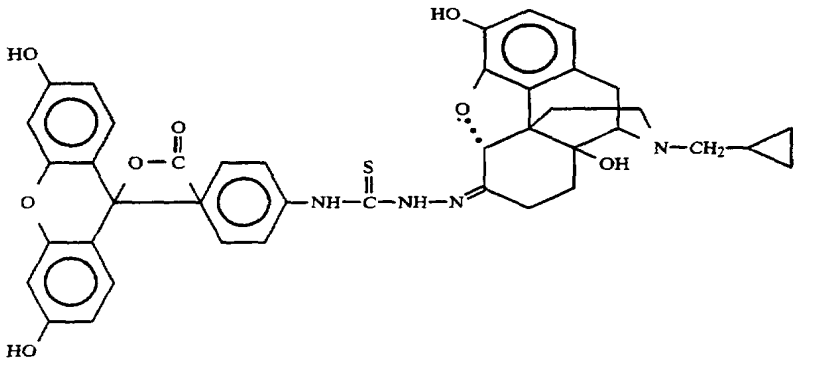
Solubility characteristics of the fluorescer (or other non-opioid moiety) may also have a bearing on the mode of administration of the weight control agent. Thus, an agent containing a highly soluble fluorescer such as G-orange may be advantageously administered orally, while a relatively insoluble agent might more usefully be administered parenterally or subcutaneously. However, within the skill of the art, most if not all of the various weight gain control agents should be subject to administration in more than one way. Particularly preferred fluorescers include substituted and unsubstituted fluoresceinyl and substituted and unsubstituted rhodaminyl. Especially preferred are fluoresceinyl, tetramethyl-rhodaminyl-B-, the residue of G-orange, and the residue of acridone. Methods for the preparation of hybrid azines and thiosemicarbazones of opioids and fluorescent moieties are described in the aforesaid application of Vera Kolb Meyers, Ser. No. 621,384.

Among the specific hybrid fluorescent/opioid antagonist compounds useful in the mothod of the invention are:

(Formula IX; 6-FN)



(Formula X; 6-FNX)



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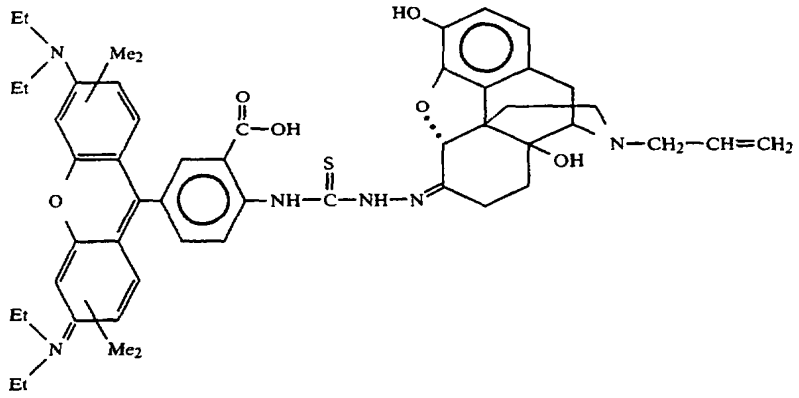
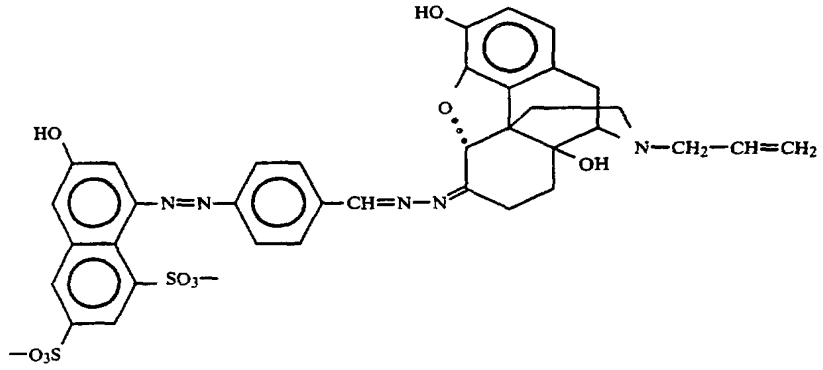


(Formula XI; 6-RN)



(Formula XII; NOZO)

The weight control agents of Formulae II and IV are novel compounds. Also novel are those compounds of Formula I which also correspond to Formula VII:

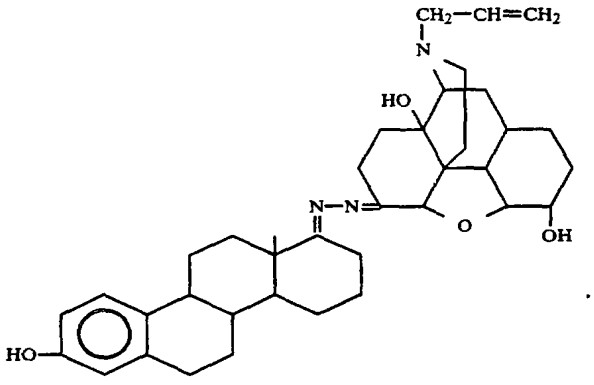
$$A^1=N-N=R^1 \qquad \text{(Formula VII)}$$

where A^1 is as defined above.

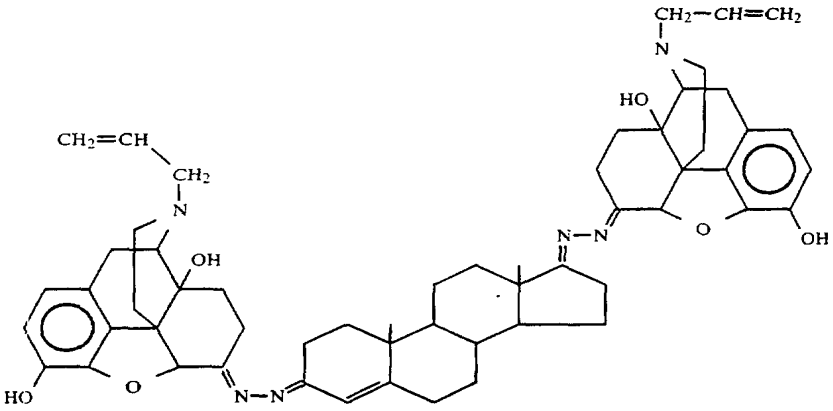
Where the compound comprising the weight control agent contains a fluorescent moiety, it is preferably of the type preferred in the compounds of Formula III, as discussed above. Where the weight control agent contains a steroid moiety, it is preferably attached to the azine nitrogen at the 3-, 6-, 11-, 16-, or 17-carbon of the steroid moiety. More preferably, it is attached at the 3-, 6-, or 17-carbon. For stability of the mixed azine, it is also preferred that there be an angular methyl group on the carbon adjacent the carbon attaching to the azine nitrogen. Suitable weight control agents may usefully be derived from estrone, androstene dione or other common steroids, preferably those which satisfy the criteria discussed above.

A hybrid azine of estrone has one opiate unit. Thus, for example, the mixed azine of naloxone and estrone corresponds to the formula:

(Formula XIII; NAL—E)



A mixed azine of a dione such as androstene dione has two opiate units and may be thought of as a bis(opioid azine) with a long rigid spacer moiety between the two opioid units. Thus the mixed azine of naloxone and androstene dione has the structure:



(Formula XIV; NAL—AD—NAL)

The mixed azines of Formula VII may be prepared by reacting an opioid antagonist free base with a hydrazone of the steroid. To carry out the reaction, a hot solution of the steroid hydrazone may be prepared and the free base of the opioid antagonist added to the solution. The progress of the resulting reaction may be followed using conventional analytical techniques, for example, thin layer chromatography. After the reaction is substantially complete, the reaction mixture is preferably quenched with H_2O, after which solid product obtained may be recovered by filtration and dried. Steric factors favor the formation of the anti-anti isomer in this synthesis. A similar method may be used to prepare the mixed diazine of an opioid antagonist and a steroid dione, such as androstene-3,17-dione, except that in the latter case the free base of the opioid is reacted with the corresponding dihydrazone of the steroid.

The novel N,N'-disubsituted thioureas of Formula IV may be prepared by reaction of a fluorescent substituted isothiocyanate with an amino substituted, typically a 6-amino substituted, opioid antagonist. Either an alpha amine or beta amne can be used. In the synthesis, the amine substituted opioid may be dissolved in a suitable solvent such as triethylamine, the isothiocyanate of the fluorescer dissoved in a solvent such as a lower alcohol, and the two solutions mixed and reacted at moderate temperature, for example, room temperature, for a time sufficient for condensation of the amine and isothiocyanate groups. Again, the progress of the reaction may conveniently be followed by thin layer chromatography. After the reaction is complete, the product may be recovered by concentrating the reaction mixture, followed by crystallizion. Addition of a lower alcohol may promete the crystallization.

Novel preparations for control of weight are provided by combining the weight control agent with other materials which facilitate its administration to a mammal. Thus, for example, where oral administration is contemplated, the weight control agent may be mixed with pharmaceutically acceptable excipients and/or adjuvants. Where parenteral or subcutaneous administration is desired, the weight gain inhibiting agent may be formulated with a suitable pharmaceutically acceptable carrier. Dosage amounts and concentrations of particular components may be readily determined by those skilled in the art.

To carry out the novel method of the invention, an effective amount of the weight control agent is administered to the mammal in any convenient manner, but preferably orally. Upon ingestion, injection, or absorption, the agent attaches to the opioid receptor sites in the gut or brain and blocks them, resulting in inhibition of weight gain. In most instances, administration of an appropriate amount of the weight control agent will result in an actual loss of weight. Because the weight control agents used in the method of the invention are long lasting, effective control of weight can be realized by the administration of modest amounts of the agent at relatively infrequent intervals. A persistent and even effect is thus achieved with minimal toxicity or other adverse side effects.

The following examples illustrate the invention.

EXAMPLE 1

Synthesis of 1-(N)-Fluoresceinyl Naloxone Thiosemicarbazone ("6-FN")

Naloxone hydrazone (0.114 grams; 0.333 millimoles) was added to a solution of fluorescein isothiocyanate (0.1351 gram; 0.347 millimoles; Sigma Chemical, St. Louis, isomer I) in tetrahydrofuran (2 ml.; Fisher Certified) and ethyl alcohol (4 ml.; 100%, histological quality). The resulting solution was stirred at room temperature until the reaction mixture contained no more naloxone hydrazone as indicated by a thin layer chromatography (TLC). The container was wrapped in aluminum foil. Thereafter, portions of ethyl alcohol and water were added to the reaction mixture. Orange crystals formed which were filtered off and dried. The structure of the crystals was determined to be that of 1-(N)-fluoresceinyl naloxone thiosemicarbazone ("6-FN") based on spectroscopic evidence and elemental analysis. Major physical and spectral characteristics of 6-FN included: Mp>300°. IR (nujol): υ1740 (C=C), 1635, 1590, 1500, 1325, 1268, 1208, 1182, 1110, 1028, 993, 952, 912, 852, 808, 722, 618 cm^{-1}. ^{1}H-NMR (100 MHz) (DMSO-d_6): δ8.442 (1H, d, J~2 Hz), δ8.034 (1H, split d, J 8 Hz, J 2 Hz), 7.251 (1H, d, J~8 Hz) (protons from the non-phenolic aromatic ring of the fluorescein moiety); 6.678, 6.602, 6.583 (8 H's, single peaks) (aromatic protons from the phenol rings of the fluorescein and naloxone moieties); 6.11-5.10 (complex signal, vinylic H's of the allyl group); 5.029 (1H, s, bridgehead H at C-5) ppm; the upfield peaks look like those of naloxone. C,H,N,S analysis: calculated: C, 65.74; H, 4.69; N, 7.67; S, 4.39. Found: C, 65.81; H, 4.64; N, 7.64; S, 4.34. Fluorescence properties of 6-FN: (a) A 10^{-6}M solution in pH 7 phosphate buffer showed an excitation maximum

at 493 nm and an emission maximum at 513 nm, as compared to 493 nm and 510-511 nm, respectively, for free fluorescein; (b) Absorption maximum 10^{-5}M solution (phosphate buffer) was at 493 nm (A=0.410; c) Quantum yield of 6 FN compared to free fluorescein was about 67% at 10^{-9}M; d) fluorescence polarization was 0.038 for 6-FN and 0.022 for free fluorescein.

EXAMPLE 2

Preparation of 1-(N)-tetramethylrhodaminyl-B-naloxone Thiosemicarbazone ("6RN")

Tetramethylrhodaminyl-B-isothiocyanate (Sigma, St. Louis; 10 mg.; $2.255\times10-5$ mol) was almost completely dissolved in a mixture of tetrahydrofuran and ethyl alcohol (approximately 3 cc), producing a wine-red solution. To this solution was added naloxazone, i.e., naloxone hydrazone, (8.5 mg.; $2.49\times10-5$ mol). After the naloxazone was added, the solution changed from wine-red to a brownish color and became cloudy. After 33 minutes of reaction, a sample of the reaction solution was removed and subjected to thin-layer chromatographic analysis. The results of this analysis did not indicate the presence of any remaining naloxazone. Approximately one hour after the addition of naloxazone, the reaction mixture was quenched by addition of water. Upon quenching, the solution warmed slightly, but no precipitate formed. Thereafter, the solution was concentrated to approximately its initial volume by removal of solvent on a rotary evaporator under mild heating. Significant foaming was observed during the concentration of the solution, and a precipitate formed which was separated by filtration. Thereafter, the mother liquor was subjected to further evaporation to one quarter of the original volume, upon which additional precipitation occurred, producing a second crop of crystals which was also removed by filtration. Both crops of crystals were dried in a vacuum oven. The product was 1-(N)-tetramethylrhodaminyl-B-naloxone thiosemicarbazone.

EXAMPLE 3

Synthesis of 1-(N)-Fluorosceinyl Naltrexone Thiosemicarbazone ("6-FNX")

Naltrexazone (0.0573 grams; 1.61×10^{-4} mol) was dissolved in a minimum amount of tetrahydrofuran and the resulting solution added (together with ethyl alcohol rinsings from the container in which the dissolution was carried out) to a stirred solution of fluoresceinyl isothiocyanate (Sigma Chemical Co., Isomer I; 0.0641 grams; 1.65×10^{-4}mol) in THF (lcc; Fisher, certified) and ethyl alcohol (2cc). As soon as the naltrexazone was added, the color of the fluoresceinyl isothiocynate solution began changing from red to orange. Reaction was carried out under agitation at room temperature while the reaction solution was protected from light with aluminum foil. After approximately one hour and 5 minutes of reaction, a small quantity of precipitate was formed and a sample taken for TLC analysis. Thereafter, additional ethyl alcohol was added until further precipitation was obtained. After one hour and 35 minutes the reaction was stopped by filtering out the solid precipitate. The solid product was washed twice with ethyl alcohol, yielding a crystallizate of 1-(N)-fluoresceinyl naltrexone thiosemicarbazone ("6-FNX") (0.0223 grams), determined by TLC analysis to be substantially pure.

Further crystalization from the mother liquor yielded an additional quantity of 6-FNX (0.0182 grams). By adding water to the remaining mother liquor, a third crop of crystalline 6-FNX was obtained (0.0410 grams). The third crop was washed with ethyl alcohol and water. Overall yield at this point was 67.9%. Further crystallization from the mother liquor provided a fourth crop of 6-FNX crystals (0.0138 grams) bringing the total yield to 79.4%.

EXAMPLE 4

1-(N)-fluoresceinyl benzophenone thiosemicarbazone ("6-FB") was synthesized in a manner comparable to the methods described in Examples 1-4.

The opioid receptor binding characteristics of 6-FN, 6-FNX, 1-(N)-fluoresceinyl oxymorphone thiosemicarbazone ("6-FO)", 6-FB, and 6-RN were assessed by their effectiveness in displacing dihydromorphine (^{3}H-DHM) from rat brain synaptosomal plasma membranes. In this assessment, radiolabeled ^{3}H-DHM (^{3}H-DHM*) was initially bound to the receptor sites on the membranes, following which the membranes were contacted with solutions of the fluorescent-labeled reagent. After removal of the reagent solution, the membranes were washed for removal of any displaced ^{3}H-DHM*. Radioactivity counting both before and after treatment with the fluorescent reagent gave an indication of displacement of ^{3}H-DHM*.

All of the fluorescent-labeled compounds except 6-FB were found effective for displacement of ^{3}H-DHM*. Increasing concentrations of the fluorescent reagents were effective for progressive displacement of the indicator ligand. Generally, the fluorescent reagents were not as effective as their parent opioids as competitors for displacement of ^{3}H-DHM*. However, 6-FN, 6-FNX, 6-FO and 6-RN showed sufficient binding activity for use in the detection and quantification of receptor sites in the synaptosomal membranes. The following IC_{50} values (concentrations necessary for 50% displacement of ^{3}H-DHM*) were determined graphically: naloxone 0.9 nM; 6-FN 20 nM, 6-RN 12 nM; naltrexohe 0.3 nM; 6-FNX 5 nM; 6-FO 20 nM. The control compound 6-FB was inactive at 10^{-6}M. The Hill coefficients were: naloxone 1.09; 6-FN 0.671; 6-RN 1.09; naltrexone 1.15; 6-FNX 0.675; 6-FO 0.989.

In order to investigate the anomalous displacement curve of 6-FN, this reagent was also subjected to the site selectivity analysis technique described by Terenius and Wahlstrom, *European Journal of Pharmacology*, Vol. 40, pp. 241–248 (1976). In this analysis, ^{3}H-DHM and ^{3}H-D-Ala^2-[Leu]enkephalin (3H-DALE) were used as indicator ligands. To provide a comparison of site selectivity, the non-peptide antagonist compound was tested for displacement of labeled opioid from a substrate which had been previously contacted with an indicator ligand solution containing DHM*(0.43nM) and cold DALE (0.43 nM). These results were compared with the displacement obtained from a substrate which had been contacted with an indicator ligand solution containing cold DHM (0.43nM) and DALE* (0.43nM). FIG. 1 is a plot of percent bound DALE* as obtained from the second displacement test versus percent bound DHM* from the first displacement test for a series of antagonist compound solutions of varying strength. In FIG. 1 it will be noted that compounds which displace the two indicator ligands differently provide a curve which is convex towards the axis of the labeled indicator ligand which is less favorably dis-

placed. The results of this test show clearly the difference between 6-FN and naloxone with respect to site selectivity. The IC50 values determined graphically from this test were: (a) with ^{3}H-DHM; naloxone 1.3 nM; 6-FN 12 nM; (b) with ^{3}H-DALE; naloxone 20 nM; 6-FN 17 nM.

EXAMPLE 5

6-FN was tested for activity on an electrically stimulated guinea pig ileum longitudinal muscle preparation. In this test, a specimen of guinea pig ileum was stretched between two wires in a nutrient solution (2.5 mil.) and stimulated supramaximally at 0.1 Hz. In the absence of opioid agonists and antagonists electrical stimulation of the muscle results in contraction.

Addition of an aqueous solution of normorphine (25 microliters; 10^{-7}M) substantially inhibited contraction of the muscle (FIG. 2). Introduction of an aqueous solution of 6-FN (10^{-6}M in 10% ethanol; 25 microliters) into the nutrient solution (final 6-FN concentration: 10^{-7}M) prior to the introduction of normorphine significantly inhibited the agonist effect of normorphine, though the effect was ten times less effective than the inhibiting effect achieved with naloxone. Washing of the ileum specimen did not readily eliminate the antagonist effect. In this respect 6-FN was similar to naloxone.

As illustrated in FIG. 2, 10^{-6}M 6-FN exhibited a partial agonist activity which was observed to be reversible with 10^{-7}M naloxone.

EXAMPLE 6

Synthesis of the Mixed Azine of Oxymorphazone and G-Orange ("OZO")

G-orange (Fluka; 76.3 mg; 0.1588 mmol) was dissolved almost completely in a mixture of water and ethanol. The G-orange was first mixed with the water (1.2 ml) after which the ethanol (0.7 ml) was added to the mixture. A solution of oxymorphazone (50.6 mg; 0.1606 mmol) in ethanol,(200 l) and a minor amount of water was prepared and added to the G-orange solution. Inclusion of a minor fraction of ethanol in the aqueous solution of G-orange, and a minor fraction of water in ethanolic solution of oxymorphazone, prevented the formation of a precipitate that might otherwise have been produced on contact of the two solutions. The mixed solution of G-orange and oxymorphazone was stirred at room temperature in the dark for 7½ hours, during which time the progress of the reaction was followed by thin layer chromotography (TLC). After 7½ hours, some solid was observed to have been formed in the flask. Additional ethanol was thereafter added to the reaction solution and the solid filtered off. The solid product was dark purple in color and was insoluble in ethanol but soluble in water. Upon evaporation of the reaction solution mother liquor, additional solid precipitated. A crude solid product obtained was analyzed for purity by both TLC and HPLC. Both methods indicated a complete absence of oxymorphazone in the product, and only a trace of G-orange.

Two solvent systems were used for TLC, the R_f values for which are indicated in Table 1. The TLC was carried out on E. Merck aluminum supported sheets (precoated TLC sheets, silica gel 60F-254, layer thickness 0.2 mm, catalogue no. 5539). The two eluents used were. System 1: chloroform: methanol: concentrated ammonium hydroxide=135:10:2 (v/v), and System II: ethanol: acetic acid: water=60:30:10 (v/v). The spots were observed in UV—254 nm; 366 nm for the fluorescent compounds. The latter were also observed in visible light.

The HPLC peak positions are given in Table 2. These analyses were done on a Laboratory Data Control instrument equipped with a C-18 bondapack analytical column from Waters. The solvent used for the HPLC was acrylonitrile 0 to 80% gradient, 0.1% ammonium acetate (pH about 5.9).

TABLE 1

Compound	R_f Values System I R_f	System II R_f	Comment
oxymorphazone	0.32	0.07	spot observed in UV (254 nm)
G-orange	0	0.59	orange spot (vis. light)
OZO	0	0	purple spot (vis. light)

TABLE 2

Peak positions in HPLC [mm]		
oxymorphazone	23.2 (syn)	20.0 (anti)
G-orange	26.5	
OZO	34.5	

EXAMPLE 7

Synthesis of the Mized Azine of Naloxone and G-Orange ("NOZO")

G-orange (Fluka; 20.0 mg; 0.0416 mmol) and naloxazone (14.1 mg; 0.0414 mmol) were reacted in a manner analogous to that described in Example 6 for the synthesis of the mixed azine of oxymorphone and G-orange The TLC and HPLC behavior was closely analogous to that reflected in the data of Tables 1 and 2 for the reaction with oxymorphazone. R_f values for NOZO in both systems 1 and 2 were zero. The HPLC peak position was at 34.0 mm but another small peak at 37.1 mm was also present. This small peak probably indicates the presence of an isomer of the azine (three isomers being possible anti-anti, anti-syn, and syn-syn).

Neither G-orange nor naloxazone were present in the NOZO product as detected by either TLC or HPLC.

EXAMPLE 8

Synthesis of fluorescein labeled 6-δ-aminonaloxone [N-(1-N-allyl-14-hydroxynordihydro-6δ-morphinanyl)-N'-fluorosceinyl thiourea;"FAN"]

6δ-amino naloxone dihydrochloride (22.0 mg; 0.0549 mmol) was dissolved in triethylamine (300 μl). Fluorescein isothiocyanate (Sigma; isomer 1; 24.4 mg; 0.0627 mmol) was dissolved in ethanol (200 μl) and triethylamine (20 μl). The two solutions were thereafter mixed and maintained at room temperature in the dark (wrapped in aluminum foil) for two hours, during which a condensation reaction occurred between the amine and isothiocyanate. The progress of the reaction was followed by TLC. After two hours, a portion of water was added to the reaction mixture and the mixture then concentrated by vaporation on a rotary evaporator. A portion of ethanol was added and crystallization resulted, producing orange crystals. Additional crystals were obtained by cooling the mother liquor. As indicated by both TLC and HPLC analysis, the product was substantially pure N-(1-N-allyl-14-hydroxynordi

hydro-6δ-morphinanyl)-N'-fluoresceinyl-thiourea. TLC and HPLC data is set forth in Table 3.

TABLE III

Compound	R_f Values System I R_f	System II R_f	Comment
6 -amino naloxone	0.045	0.31	spot observed in UV (254 nm)
FITC + Et_3N + HCl	0	0.81	orange spot
FAN	0	0.58	orange spot

Using the method of Example 4, various weight control agents of the invention were tested for displacement of ^{3}H-dihydromorphine from opioid receptors in rat brain membrane preparations. For purposes of comparison, the displacement tests were also run on naloxone ("NAL"), naltrexone ("NALT") and oxymorphone ("OXY"), and the mixed azine of oxymorphone and G-orange ("OZO"). The data was analyzed, using the program LIGAND of Munson and Rodbard. The results of these displacement tests are summarized in Table 4.

TABLE IV

COMP	Kd nM	% STD. ERROR	MEANSQ.	SUMSQ.
DHM	3.1	10.9	3.13	42.3
NAL	1.6	16.4	4.44	38.9
6-FN	11.5	11.5	1.44	18.0
6-RN	5.3	14.7	2.91	34.2
6-FAN	18.9	16.6	1.41	16.5
NAZO	120.5	14.5	1.35	15.9
OXY	1.1	18.4	1.22	10.7
6-FO	12.8	16.7	0.94	11.0
OZO	37.0	16.3	5.09	59.8
NALT	0.4	15.2	0.81	6.1
6-FNX	2.6	16.9	4.93	52.5

EXAMPLE 10

To determine the persistance of binding of certain weight control agents of the invention to opioid receptor sites, the extent of dissociation of the opioid from the receptor site effected by washing was determined in accordance with the method described by E. H. Hahn et al, *J. Neuroscience*, 2, p. 572 (1982). Inhibition of the binding of ^{3}H-DHM by the weight control agent was determined both prior to washing and after three wash cycles. The tests were conducted in 50 nM Tris HCl pH 7.5 buffer. The results of these tests are set forth in FIG. 3.

Similar tests were run in HEPES buffer and the effect on ^{3}H-DHM displacement determined before washing, after three washes, and after five washes. The results are set forth in FIG. 4.

EXAMPLE 11

Synthesis Of A Mixed Azine Of Estrone And Naloxone ("NAL-E").

The mixed azine was prepared by reacting naloxone free base with estrone hydrazone in a one-to-one molar ratio. To prepare the free base, naloxone hydrochloride (Endo) was dissolved in water and poured into a separatory funnel containing dilute borax solution and chloroform. The resulting mixture was extracted three times with chloroform. The combined chloroform extracts were dried and evaporated, giving the naloxone free base as a white crystalline solid in 100% yield.

Estrone hydrazone was prepared in the manner described by Dandliker et al, *Cancer Research*, Vol. 38, p. 4212 (1978). Estrone hydrazone was crystallized out of the reaction mixture (melting point greater than 270° C). Its ^{13}C-NMR revealed only one (anti) isomer at the 17-carbon.

Estrone hydrazone (0.0757 g., 2.66×10^{-4} mol) was dissolved in boiling ethanol (5 ml; 100%). To this hot solution naloxone free base (0.0881 gr., 2.69×10^{-4} mol) was added, and the resulting mixture was boiled briefly and then left to stir at room temperature in the dark. The progress of the reaction was followed by TLC, using two different methods for detection of spots: observing them under UV light; and observing the color development after the TLC plate was sprayed with 50% sulfuric acid solution and heated to over 100° C. Naloxone shows up very strongly in the UV, while estrone hydrazone shows up only faintly. The product mixed azine, which has a higher R_f value than either naloxone or estrone hydrazone, shows up very strongly in the UV. By using the color development method, estrone hydrazone (red) and the mixed azine product (orange) can be detected very easily, while naloxone does not show up at all. After only traces of naloxone and estrone hydrazone could be detected by TLC, the reaction was quenched by pouring the reaction solution into ice. The white solid thus formed was filtered off and dried in a vacuum oven, giving 0.1303 g (82.3% yield) of the mixed azine of estrone and naloxone (melting point greater than 120° C Dec.). The product was recrystallized from chloroform and gave a satisfactory C, H, N analysis. Its IR, ^{1}H NMR and ^{13}C-NMr were in agreement with the proposed structure. The ^{13}C-NMR revealed that the azine bond is configurationaly pure, i.e., anti-anti. The MS (electron impact) did not give the parent peak as opposed to, for example, the hydrazones of naloxone, naltrexone and oxymorphone.

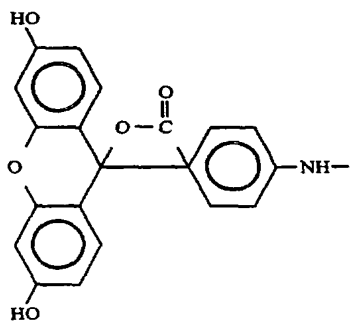
In view of the above, it will be seen that the several objects of the invention are achieved and other advantageous results attained.

As various changes could be made in the above methods and products without departing from the scope of the invention, it is intended that all matter contained in the above description or shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

We claim:

1. A method for controlling weight in a mammal comprising administering to said mammal a weight control agent for inhibiting weight gain, wherein said agent comprises a compound corresponding to he formula:

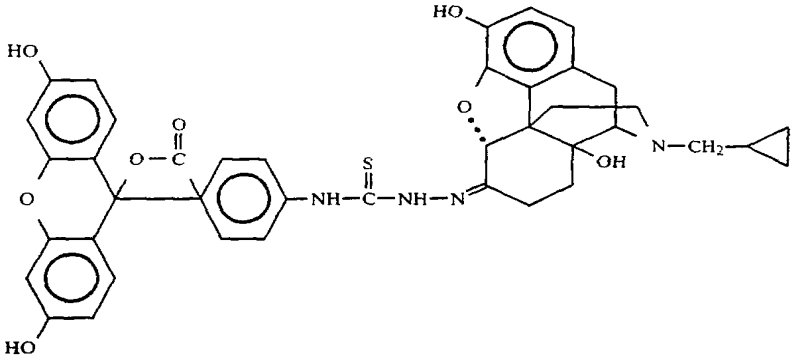
(Formula IX)



-continued

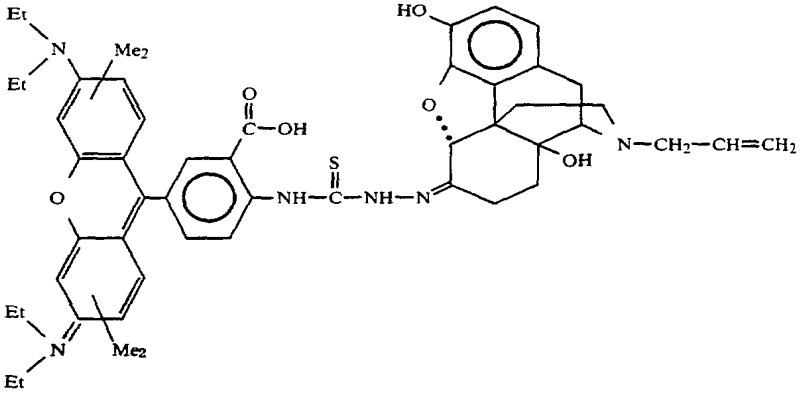
agent comprises a compound corresponding to the formula:

(Formula X) (6-FNX)



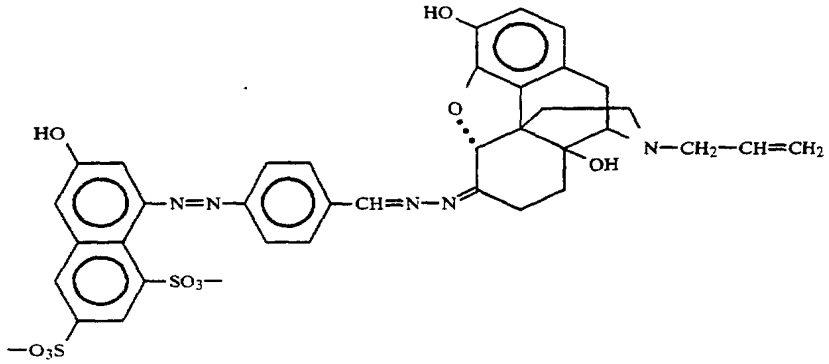
3. A method for controlling weight in a mammal comprising administering to said mammal a weight control agent for inhibiting weight gain, wherein said agent comprises a compound corresponding to the formula:

(Formula XI) (6-RN)



4. A method for controlling weight in a mammal comprising administering to said mammal a weight control agent for inhibiting weight gain, wherein said agent comprises a compound corresponding to the formula:

(Formula XII) (NOZO)

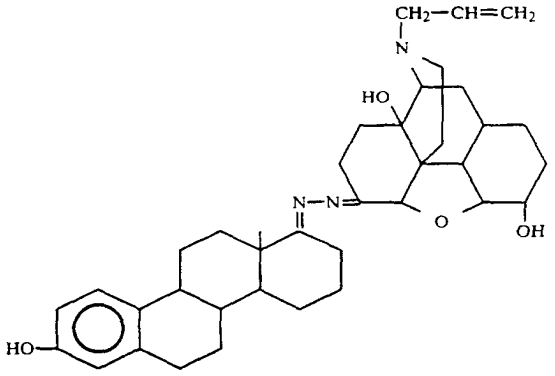


2. A method for controlling weight in a mammal comprising administering to said mammal a weight control agent for inhibiting weight gain, wherein said

5. A method for controlling weight in a mammal comprising administering to said mammal a weight control agent for inhibiting weight gain, wherein said

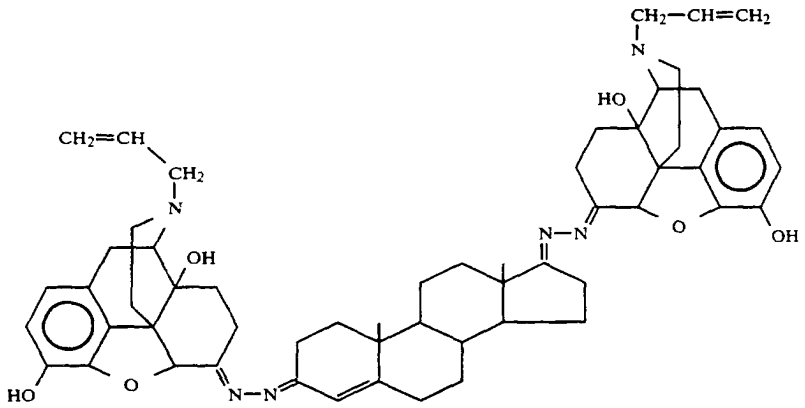
agent comprises a compound corresponding to the formula:

(Formula XIII) (NAL—E)



6. A method for controlling weight in a mammal comprising administering to said mammal a weight control agent for inhibiting weight gain, wherein said agent comprises a compound corresponding to the formula:

(Formula XIV) (NAL—AD—NAL)



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